THE DESTRUCTION OF LUMINOUS BACTERIA BY HIGH FREQUENCY SOUND WAVES

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In a previous communication (1928) we have described the dimming of the light of luminous bacteria exposed to high power high frequency sound waves, generated by the apparatus of Wood and Loomis (1927). This consists of a 2-kilowatt oscillator giving a maximum of 50,000 volts across a quartz crystal in an oil bath. A test tube containing the bacteria suspended in sea water touches the surface of the oil, and the sound waves generated in the vibrating quartz crystal by the piezo-electric effect, pass through the oil, the glass of the test tube and the sea water suspension of the bacteria. Considerable energy is introduced, which heats the media, so that in the original experiments, using a frequency of 406,000, the bacteria were first cooled to 1.5° and rayed until their temperature reached 21.5°. This takes only a few minutes, but in this time the bacterial light is perceptibly dimmed, although not extinguished.

If a low powered oscillator is used and the bacteria mounted under a cover slip on the crystal and placed on the microscope stage without an oil bath, as described in our previous paper (1928), one can observe great agitation of the bacteria but no breaking up, such as happens to infusoria. The bacteria are carried into nodes of a complex wave pattern and remain unharmed, but the convection currents in a large test tube prevent the formation of permanent nodal accumulations of the bacteria.

In our recent experiments the bacteria (rod shaped *Bacillus Fisheri*)[1] were placed in a large test tube containing a glass coil

[1] I express sincere thanks to my students, Dr. S. E. Hill, Mr. C. S. Shoup and Mr. E. Holcomb for growing and supplying the luminous bacteria used in these experiments.—E. N. H.

through which ice water was circulating to keep down the temperature, and with another glass tube opening under the surface of the bacterial suspension, through which air, filtered through sterile cotton wool, was bubbled to keep the suspension stirred. The top of the test tube was cotton plugged and sterilized and the whole experiment so carried out as to prevent contamination with foreign bacteria. The high power oscillator was used with a frequency of 375,000 per second. It was found that even with ice water cooling of the suspension, the oil bath became so hot that in thirty minutes the temperature of the bacteria had risen to 35°C. As this is sufficient to cause near extinction of the light of the bacteria without exposure to sound waves, the oil bath in later experiments was also cooled with coils of lead tubing. The lead tubing and all metal parts near the high tension field must be grounded to prevent sparking.

After the exposure to sound waves the bacteria were inoculated on agar slants and Petri dishes to determine if all were killed. A number of experiments were carried out, as follows:

A1. Rayed for thirty minutes, when the suspension had almost completely cleared, an indication that the bacteria are broken up, and the light had disappeared, but this might be due to heating, as the temperature had risen to 35°C.

A2. Same procedure and results as A1.

A3. The same bacterial emulsion was heated to 35°C. in a water bath for thirty minutes and then cooled. It did not clear, but the bacterial luminescence had disappeared.

Platinum loop transfers of A1, A2, and A3, were made to agar slants as well as from a control suspension of bacteria unheated. After sixteen hours the control showed good luminescence and growth while A1, A2 and A3 showed no luminescence or growth. After forty-eight hours, A3, the heated tube, showed good luminescence from about one hundred colonies while A1 and A2 showed luminescence and growth from twelve and three colonies respectively. Evidently the heating had seriously injured the bacteria and the raying plus the heating had killed nearly all. There were no growing colonies without luminescence. This experiment indicates that the bacteria are mostly killed by one

half hour raying, but does not tell whether the luminescence can be completely extinguished, as the temperature rose too much.

In another experiment (B1) the bacteria were rayed for an hour and the temperature kept below 19°C. by cooling the oil as well as the bacterial suspension. The rayed bacteria showed no growth or luminescence when plated on the Petri dishes or when inoculated on agar slants, while the control inoculations grew and luminesced brilliantly. The luminescence dims rather quickly on raying but the last trace of light persists for something like twenty to thirty minutes.

Examined under the microscope the rayed suspensions show practically no bacteria while the controls are full of actively moving individuals. As indicated by the clearing of the suspensions the bacteria must be thoroughly torn up and cytolysed as they are when placed in water. The silkiness, due to optical effects of needle particles, observed when normal luminous bacteria suspended in sea water are gently agitated, had disappeared in the rayed tubes. Another experiment (B2), like the above, but in which the temperature was kept below 15°C., gave identical results.

C1. Finally a very dense emulsion of luminous bacteria in sea water was rayed for ninety minutes and loop samples inoculated on agar slants every five minutes. The temperature was kept below 16°C. throughout the experiment. The luminescence gradually dimmed until it was faint (forty-five minutes) and only a just perceptible luminescence remained after ninety minutes raying. After fourteen hours the inoculations made up to fifty minutes raying all showed good luminescent growth, the fifty-, sixty-, and seventy-minute inoculations a few colonies, and the eighty-, and ninety-minute inoculations no luminescence. After twenty-four hours all inoculations showed good luminescent growth except the ninety-minute with one colony and after thirty-six hours, all inoculations showed some luminescent growth. The raying had evidently killed most of the bacteria, only a few more resistant ones persisting with their power of growth retarded.

The rayed emulsion had partially cleared and lost most of its silky appearance on gentle shaking, characteristic of rod and

needle particles in suspension and examined under the microscope showed very few rods but many small granules, like fragments of bacteria. There is also a coagulum of fiber-like material which collects on the side of the test tube and cooling tubes, in all the rayed suspensions. This is not cotton fiber as it gives a strong xanthoproteic test and is undoubtedly composed of the debris of the bacteria stuck together in some peculiar manner under the influence of the sound waves. It never appeared in control unrayed tubes of bacterial suspension through which air was bubbled as in the experimental tubes.

It should again be emphasized that the conditions of the raying are such in these experiments, namely, a round bottomed tube and constant agitation, that no standing waves can be set up and consequently the bacteria cannot be thrown into nodes, where they remain unharmed, as happens in capillary tubes or spaces.

In order to see whether needle crystals could be broken up by raying, a suspension of benzopurpurin in water was rayed for one hour, with ice water cooling. The needle crystals, easily visible under high power of the microscope, are broken apart into shorter lengths but not sufficiently so as to abolish the silkiness normally exhibited by these solutions. A silky soap solution with needle crystals was not affected by one hour's raying. Perhaps we should not expect such needle particles whose consistency and rigidity undoubtedly differ from that of bacteria to behave in the same way.

In conclusion we can state that, under proper conditions of raying, luminous bacteria can be broken up and killed by sound waves of approximately 400,000 frequency and the solutions sterilized, but that the method is not one of any practical or commercial importance because of the expense of the process.

REFERENCES

HARVEY, E. N., AND LOOMIS, A. L. 1928 Nature, 121, 622–624.
WOOD, R. W., AND LOOMIS, A. L. 1927 Phil. Mag., 4, 417–436.